

Amendments to the Specification:

On page 1, please delete the paragraph before “FIELD OF THE INVENTION”, and replace it with the following paragraph:

Related Applications

This application is a divisional of co-pending U.S. Application Serial No. 10/205,115, filed July 25, 2002, which is a continuation of U.S. Application No. Serial No. 08/325,540, filed October 18, 1994, now abandoned, which is a continuation of U.S. Application Serial No. 07/919,851, filed July 31, 1992, now abandoned, which is a continuation-in-part of U.S. Application Serial. No. 07/773,042, filed October 10, 1991, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 07/740,501, filed August 5, 1991, now abandoned. Each of these applications is incorporated herein by reference.

After page 186, please add the following paragraphs:

Preparation of the uridine 5'-O-aryl ester 91

A mixture of acid 82 (5 mmol), alcohol 74 (5 mmol), and EDC (6 mmol) in 50 mL of CH₂Cl₂ is stirred at room temperature until the starting material is consumed. The solution is washed with water (2 x 30 mL), the aqueous phases are washed with CH₂Cl₂ (2 x 50 mL), the organic phases are dried over anhydrous MgSO₄, and the solvent is evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of the monoacid 92

Diester 91 (5 mmol) is dissolved in ethyl acetate (100 mL), 5% Pd-C (10% by weight) is added, and the mixture is stirred under a hydrogen atmosphere until the starting material is consumed.

The catalyst is filtered out using a pad of Celite, and the catalyst is rinsed with ethyl acetate (100 mL). The solvent is evaporated *in vacuo*, and the product is used without further purification.

Example 22: Preparation of the Hapten of the Prodrug in Example 20, the cyclobutanol substituted by a 5'-O-acyl uridine, compound 100.

Refer to Figure 34 for the bold numbered compounds in this Example.

Cyclobutenedione 93, prepared following a literature procedure, is converted, after several steps, to the aminocyclobutanediol 97. Selective N- and O-acylation reactions and deprotection gives the cyclobutanol hapten 100.

In detail, the synthesis is as follows:

3-Hydroxy-4-methyl-3-cyclobutene-1,2-dione (93)

Compound 93 can be synthesized using the procedure of Bellus, D., et al., *Helv. Chin. Acta* 63 (1980):1130-1140.

3-tert-Butyldimethylsiloxy-4-methylcyclobutene-1,2-dione (94)

Imidazole (11 mmol) is added to a solution of compound 93 (5 mmol) and tert-butyldimethylchlorosilane (5.5 mmol) in 5 mL of DMF cooled by an ice bath. The mixture is allowed to warm to room temperature. After 16 hours, the mixture is poured into water (50 mL) and extracted with ether (3 x 50 mL), the organic phases are washed with brine (50 mL) and dried over anhydrous MgSO₄, and the solvent is evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of cyclobutenediol 95

Sodium borohydride (20 mmol) is added to a solution of compound 94 (5 mmol) in 50 mL of ethanol cooled by an ice bath. After the starting material is consumed as observed by TLC, the

mixture is poured into water (100 mL) and extracted with ether (4 x 75 mL), the organic phases are washed with brine (50 mL) and dried over anhydrous MgSO₄, and the solvent is evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of aminocyclobutanediol 96

Tetrabutylammonium fluoride (1.0M solution in THF, 6 mmol) is added to a solution of compound 95 (5 mmol) in 50 mL of THF cooled by an ice bath. After 30 minutes, dibenzylamine (20 mmol) is added. After an additional 15 minutes, sodium cyanoborohydride (30 mmol) is added. After an additional 2 hours, the mixture is poured into water (100 mL), the pH is adjusted to 10, the mixture is extracted with ether (4 x 75 mL), the organic phases are washed with brine (50 mL) and dried over anhydrous Na₂SO₄, and the solvent is evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of aminocyclobutanediol 97

A mixture of 5% Pd-C (10% by weight) and amine 96 (5 mmol) in 20 mL of methanol is stirred at room temperature under an atmosphere of hydrogen until the starting material is consumed as observed by TLC. The catalyst is filtered out through a pad of Celite, washing with 150 mL of additional MeOH. The solvent is evaporated *in vacuo*, and the resulting oil is used without further purification.

Preparation of amide 98

DMAP (6 mmol) is added to a solution of amine 97 (3 mmol) and thiazole ester 60 (3 mmol) in 15 mL of CH₂Cl₂. After no further progress in the reaction occurs as observed by TLC, the mixture is poured into water (50 mL) and extracted with ethyl acetate (3 x 50 mL), the organic phases are washed with brine (50 mL) and dried over anhydrous MgSO₄, and the solvent is

evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of ester 99

A mixture of acid 92 (1 mmol), alcohol 98 (1 mmol), and EDC (1.2 mmol) in 10 mL of CH₂Cl₂ is stirred at room temperature until the starting material is consumed. The solution is washed with water (2 x 20 mL), the aqueous phases are washed with CH₂Cl₂ (2 x 20 mL), the organic phases are dried over anhydrous MgSO₄, and the solvent is evaporated *in vacuo*. The residue is purified by flash chromatography to give the product as a colorless oil.

Preparation of cyclobutanol hapten 100

Trifluoroacetic acid (2 mL) was added to a mixture of compound 99 (1 mmol) and anisole (1 mL) in 10 mL of CH₂Cl₂ cooled by an ice bath. The mixture is allowed to warm to room temperature, and after 1 hour, the volatile components are evaporated *in vacuo*. The residue is used for linking to a carrier protein without further purification.

Example 23: Adriamycin & Melphalan prodrugs

Adriamycin and daunomycin are anthracycline anti-tumor antibiotics were found to inhibit DNA synthesis via intercalation (Di marco, A., et al., Biochem. Pharmacol 20 (1971):1323-1328). It was demonstrated that these compounds intercalate with base pair through chromophore interaction and an electrostatic interaction between the amino group of the sugar residue (daunasamine) and the phosphate group of DNA (Di marco, A., et al., Cancer Chemoth. Rep., Vol 6 No 2 (1975):91-106).

It was demonstrated that derivatizing the amino group via the amide bond formation (Chandra, P., Cancer Chemoth. Rep. (1975):115-122), by amino acids, peptides or other carboxilic acids decreased the toxicity of these compounds (Levin, Y., Febs Letters 119-122).

Example 23: Preparation of adriamycin pro-drug, aroyl amide compound 103.

Refer to fig. 27 for the bold numbered compounds in this example.